PHYLOGENETIC ANALYSES OF THE *GAYLUSSACIA FRONDOSA* COMPLEX (ERICACEAE: VACCINIEAE) BASED ON MOLECULAR AND MORPHOLOGICAL CHARACTERS

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ABSTRACT

The three closely related species of blue huckleberries, i.e., *Gaylussacia frondosa*, *G. tomentosa*, and *G. nana*, which make up the *Gaylussacia frondosa* complex (sect. *Decamerium*, subsect. *Frondosae*), have been variously circumscribed and classified by North American systematists. This study aims to resolve the species relationships of the *Gaylussacia frondosa* complex through phylogenetic analyses of morphological characters and nuclear ribosomal internal transcribed spacer (nrITS) sequences. *Gaylussacia nana* forms a distinct clade with support in all analyses. Contrary to some earlier treatments, our results strongly support distinguishing *G. nana* from *G. tomentosa*, although a few putative hybrids between these species were discovered. The monophyly of *G. tomentosa* is not strongly supported, but is upheld in the morphology-based analysis and the combined (nrITS + morphology) analysis, and a *G. frondosa* + *G. tomentosa* clade is strongly supported in the nrITS and combined analyses. In contrast, the morphological topology groups *G. frondosa* with *G. nana*. None of the analyses support a *G. frondosa* clade, but the specimens representing *G. frondosa* do form a clade in some of the most parsimonious trees in the combined analysis, while in others they constitute a paraphyletic complex (in a clade with *G. tomentosa*). Chromosome counts by previous workers indicate that *G. tomentosa* is tetraploid, whereas *G. frondosa* and *G. nana* are diploid. *Gaylussacia frondosa* (in contrast to *G. tomentosa* and *G. nana*) tends to be a taller plant and has a more northern distribution. Based on this evidence, we suggest that all three taxa be considered distinct species (each likely representing a separate evolutionary lineage).

Key Words: *Gaylussacia frondosa, G. tomentosa, G. nana, Ericaceae, Gaylussacia sect. Decamerium*

RESUMEN

Las tres especies íntimamente relacionadas de arandaneras, i.e., *Gaylussacia frondosa*, *G. tomentosa*, y *G. nana*, que constituyen el complejo *Gaylussacia frondosa* (sect. *Decamerium*, subsect. *Frondosae*), han sido circunscritas y clasificadas variadamente por los sistemáticos norteamericanos. Este estudio trata de resolver las relaciones entre las especies del complejo *Gaylussacia frondosa* mediante análisis filogenéticos de caracteres morfológicos y secuencias del espaciador nuclear ribosómico interno (nrITS). *Gaylussacia nana* forma un clado distinto con soporte en todos los análisis. Contrariamente a algunos tratamientos previos, nuestros resultados soportan fuertemente la distinción entre *G. nana* y *G. tomentosa*, aunque se encontraron algunos híbridos putativos entre estas especies. La monofilia de *G. tomentosa* no está fuertemente soportada, sino que está apoyada en el análisis morfológico y el combinado (nrITS + morfología), y un clado *G. frondosa* + *G. tomentosa* está fuertemente soportado en los análisis de nrITS y los combinados. En contraste, la topología morfológica agrupa *G. frondosa* con *G. nana*. Ninguno de los análisis soporta un clado de *G. frondosa*, pero los especimenes que representan *G. frondosa* forma un clado en alguno de los árboles más parsimoniosos del análisis combinado, mientras que en otros constituye en complejo parafilético (en un clado con *G. tomentosa*). Los recuentos cromosomáticos realizados por investigadores anteriores indican que *G. tomentosa* es tetraploide, mientras que *G. frondosa* y *G. nana* son diploides. *Gaylussacia frondosa* (en contraste con *G. tomentosa* y *G. nana*) tiende a ser una planta más alta y tiene una distribución más septentrional. Basados en estas pruebas, sugerimos que los tres taxa sean considerados especies distintas (cada una representando probablemente una línea evolutiva separada).

Gaylussacia Kunth is a New World genus of 53 species of mainly understory shrubs occurring in mesic to xeric woodlands and shrublands, as well as acidic bogs. The genus is differentiated from the closely-related *Vaccinium* L. by ten-locular ovaries, drupaceous fruits containing ten pits, the presence of resin glands on the leaves (except for *G. brachycera* (Michx.) A. Gray), and the lack of staminal spurs (Duncan & Brittain 1966; Luteyn et al. 1996; Palser 1961; Wood 1961). However, the phylogenetic relationships between members of the two genera are still uncertain (Kron et al. 2002b). *Vaccinium* was shown to be non-monophyletic as traditionally circumscribed, with *Gaylussacia* nested within it (Kron et al. 2002a). These results are corroborated by the ambiguous generic identity of *G. brachycera*, which, in addition to being eglandular and similar in form to *Vaccinium* species, has been shown to be sister to the rest of *Gaylussacia* (Floyd 2002). Finally, some *Vaccinium* species have ovaries that are pseudo-10-locular and, therefore, resemble the 10-locular ovaries of *Gaylussacia* (Vander Kloet & Dickinson 1992).

Gaylussacia usually has been divided into three sections (following Sleumer 1967): section *Vitis-idaea* Drude (only *G. brachycera*), section *Gaylussacia* (ca. 47 species, mainly South American; plants mostly evergreen, with stalked glands on the leaves), and the North American section *Decamerium* Torr. & A. Gray (ca. five species; plants deciduous, with sessile glands on the leaves). The last is the focus of this study. All three sections are represented in eastern North America: *G. brachycera*, four of the species of section *Gaylussacia* (*G. mosieri* Small, *G. dumosa* (Andr.) A. Gray, *G. orocola* (Small) Camp, *G. bigeloviana* (Fernald) Sorrie & Weakley; see Sorrie & Weakley, 2007, although the latter two entities are often included within an expanded *G. dumosa*), and all five species of section *Decamerium* [*G. frondosa* (L.) Torr. & A. Gray, *G. tomentosa* (A. Gray) Pursh ex Small, *G. nana* (A. Gray) Small, *G. ursina* (Curtis) Torr. & A. Gray, and *G. baccata* (Wang.) K. Koch]. For more information on the history and systematics of these groups see Camp (1935), Wood (1961), Luteyn et al. (1996), Floyd (2002), and Sorrie and Weakley (2007).

A phylogenetic analysis of *Gaylussacia* had not been carried out prior to that of Floyd (2002). In her morphology-based analysis, which included most of the species of the genus, the monophyly of sects. *Decamerium* and (of course) *Vitis-idaea* were supported, but sect. *Gaylussacia* was paraphyletic. All three traditional sectional divisions were weakly to moderately supported by cpDNA (*trnL-trnF*) data, as well as in analyses combining morphological and molecular data, but nrITS sequences alone did not support the monophyly of either sect. *Decamerium* or sect. *Gaylussacia*. Molecular data were lacking for most species of sect. *Gaylussacia* and relationships within this large clade remain poorly understood. Floyd's (2002) analysis also did not clarify the placement of *G. brachycera*; neither did it fully clarify species delimitations and relationships within sect. *Decamerium*.

Camp (1941) recognized three subsections within sect. *Decamerium*, two of which, subsects. *Baccatae* and *Ursinae*, are monotypic, i.e., *Gaylussacia baccata* and *G. ursina*, respectively. The remaining three taxa, i.e., *G. frondosa*, *G. tomentosa* and *G. nana*, were placed in his subsection *Frondosae*. The taxa within the *Frondosae* group have had a confusing taxonomic history and have been variously circumscribed. Elliott (1821) and Chapman (1889) recognized only *G. frondosa*. Radford et al. (1964) recognized only *G. frondosa*, but included two varieties: i.e., *G. frondosa* var. *tomentosa* A. Gray and var. *frondosa*; Wunderlin and Hansen (2003) did the same, recognizing only var. *tomentosa* as occurring in Florida. Gray (1878) was the first author to recognize *G. frondosa* var. *tomentosa*, and he also recognized *G. frondosa* var. *nana* A. Gray. Harper (1906) treated the members of subsect. *Frondosae* as a single species with three varieties, i.e., *G. frondosa* var. *frondosa*, var. *tomentosa*, and var. *nana*. Sleumer (1967) also treated the subsection as a single species, but recognized *G. frondosa* var. *polycodioides* Camp and f. *glaucophylla* Camp (both pertaining to northern plants usually treated within var. *frondosa*). Small (1897, 1933), Camp (1935, 1941), Wood (1961), Duncan and Brittain (1966), and Luteyn et al. (1996) treated the subsection as three separate species (*G. frondosa*, *G. tomentosa*, and *G. nana*). Most recently, Floyd (2002) treated the subsection as one species with three varieties. However, she suggested that var. *nana* perhaps should be considered a separate species, citing a possible lineage sorting or hybridization event as being responsible for its incongruent placement in her nrITS and cpDNA trees. Yet, as noted by Floyd, such problems could also merely be due to inadequate data, possibly

resulting in poor resolution in her cladograms. She recommended more research in order to resolve species limits within the *Frondosae* group.

Field observations (over many years, by W.S. Judd) of the notable and seemingly consistent morphological differences between the sympatric *Gaylussacia tomentosa* and *G. nana* in Florida motivated the present study (Fig. 1). The purpose of this study is to resolve the phylogenetic relationships within this subsection, i.e., the *G. frondosa* complex, by focusing on plants identified as *G. frondosa*, *G. tomentosa* and *G. nana*. We sought to determine appropriate species limits within the *G. frondosa* complex, based on phenetic, evolutionary, diagnostic, and apomorphic species concepts (Davis & Nixon 1992; Donoghue 1985; de Queiroz 2007; Judd et al. 2007; Mishler 1985; Mishler & Theriot 2000; Wheeler & Platnick 2000; Wiley & Mayden 2000).

MATERIALS & METHODS

Taxon sampling and field work.—Voucher material representing the *Gaylussacia frondosa* complex was collected by M.T. Gajdeczka and W.S. Judd from numerous localities in Florida, Georgia, South Carolina, and North Carolina in the spring and summer of 2007 in order to estimate genetic diversity (Table 1; all deposited at FLAS). *Gaylussacia ursina* and *G. baccata* were also collected, and were selected as outgroups based on the traditional classification of section *Decamerium* (Camp 1941) and the recent phylogeny of Floyd (2002). Leaf material for DNA extraction of field-collected specimens was preserved in silica gel. DNAs derived from herbarium collections also were included in the study to supplement the number of evolutionary units in the analyses, especially of *G. frondosa*, as well as outgroup taxa. One collection of *G. dumosa* (of section *Gaylussacia*) was included as a more distant outgroup.

Morphology.—Potentially phylogenetically informative morphological characters were selected after careful consideration of the pattern of variation seen in material at the University of Florida Herbarium of the Florida Museum of Natural History (FLAS), field observations (of M.T. Gajdeczka and W.S. Judd), and previous taxonomic work on the genus (Duncan & Brittain 1966; Luteyn et al. 1996; Floyd 2002). Seventy qualitative and quantitative morphological characters were initially assessed; all above ground organs were examined, including both vegetative and reproductive features, with an emphasis on density, distribution, and form of the hairs (both glandular and non-glandular). From the initial list of characters observed, 20 were selected as potentially phylogenetically informative and were included in the matrix for analysis (see Tables 2, 3).

Unicellular hair presence, density and length and resin gland presence, density and width have been considered especially useful in distinguishing species of *Gaylussacia* (Floyd 2002; Luteyn et al. 1996), and thus these were included in the analyses. Most of the initial hair and gland measurements could be grouped into character-clusters, which showed identical or very similar patterns of variation among taxa. In such cases, a single character (from each group of highly correlated characters) was selected for inclusion in the phylogenetic analyses. However, measurement of the length of the longest hair and average hair length of the adaxial leaf surfaces (chars. #5 and 6; see Table 2) were both included in order to take into account the observed variation in the range of hair length between specimens.

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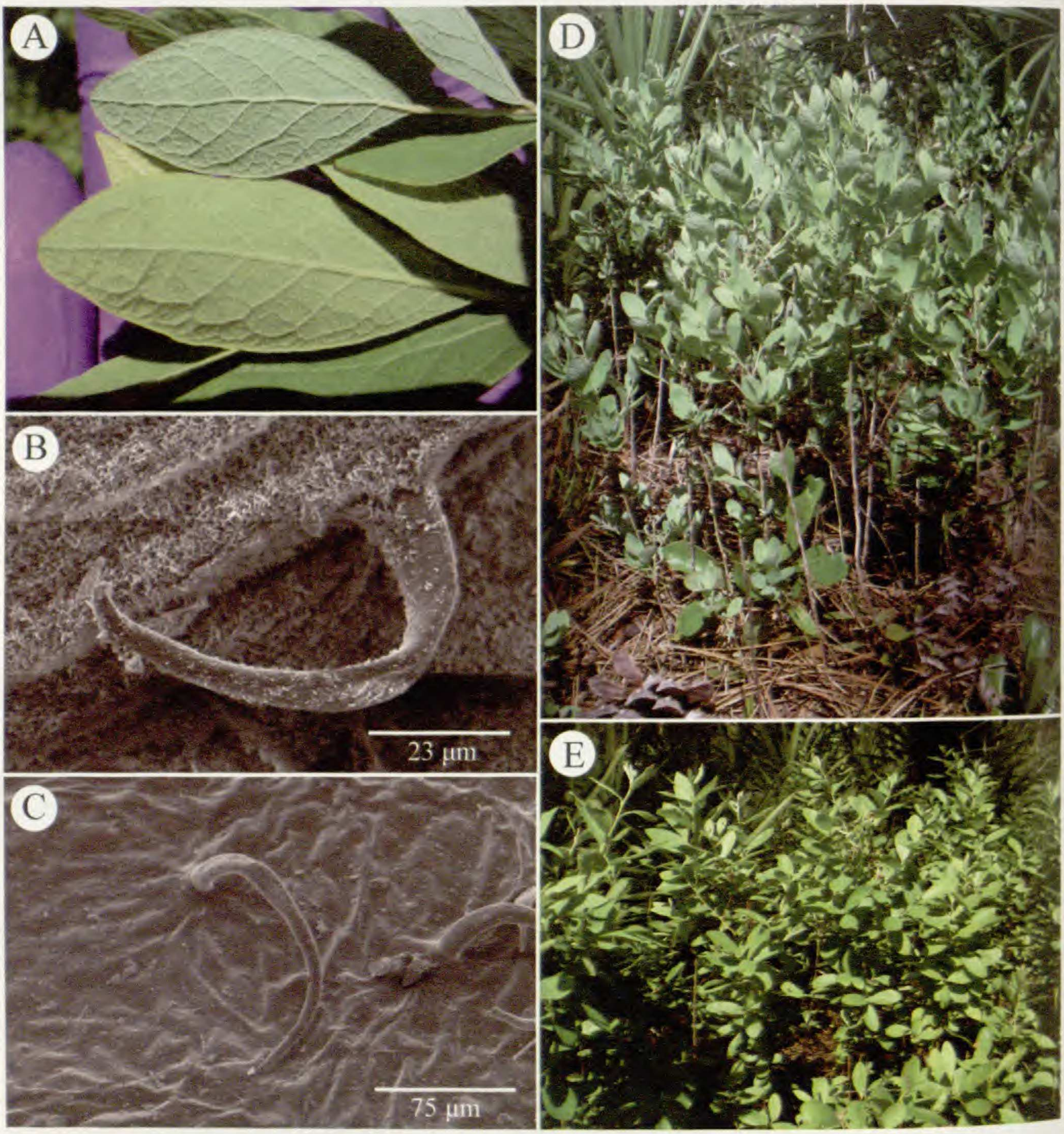

Fig. 1. *Gaylussacia nana* (top of A, B, D) and *G. tomentosa* (bottom of A, C, E). A. The glaucousness (gray coloration) of the abaxial leaf surface of *G. nana* compared to *G. tomentosa*. B&C. SEM image of glaucous abaxial surface of the leaf of *G. nana* (B) and non-glaucous abaxial leaf surface of *G. tomentosa* (C). D. Habit of *G. nana*. E. Habit of *G. tomentosa*. Photographs A, D and E, from Morningside Nature Center, Alachua Co., FL, by W.S. Judd; photographs B and C by G.M. Ionta.

across taxa (and thus could not be delimited into states). Characters not observed for particular species were scored as missing values, as were situations where a character was considered "not applicable" in a particular taxon, and coded as "?" (Table 2).

DNA extraction, amplification and sequencing.—Leaves and flowers dried in silica-gel were used for DNA extraction of field collected material. A modified version of the 2x CTAB procedure (Doyle & Doyle 1987) was used, as described in Whitten et al. (2007) with the addition of proteinase K (5 units) to the extraction buffer instead of 2-mercaptoethanol. Precipitated DNA pellets from extractions of field-collected material were resuspended in 200 µL of Tris-EDTA (TE) buffer. Small fragments of leaf tissue were removed

TABLE 1. Specimens sampled for the morphological and nrITS analyses. All vouchers deposited at FLAS.

Taxon	Voucher	Collection Location	ITS-GenBank
G. baccata	*A. Vascott 405*	King & Queen Co., VA	NA
G. baccata	*E.W. Wood 4222*	Clinton Co., PA	FJ985237
G. baccata	*W.S. Judd 1657*	Barnstable Co., MA	NA
G. baccata	*W.S. Judd 716*	Norfolk Co., MA	FJ985236
G. baccata	*M. Gajdeczka 50*	Burke Co., NC	FJ985196
G. dumosa	*M. Gajdeczka 18*	Alachua Co., FL	FJ985199
G. frondosa	*A.B. Pittman 05090108*	Orangeburg Co., SC	FJ985228
G. frondosa	*A.B. Pittman 053009303*	Aiken Co., SC	FJ985227
G. frondosa	*G.W. Seckinger, Jr. 271*	Georgetown Co., SC	FJ985226
G. frondosa	*J.B. Nelson 16473*	Richland Co., SC	NA
G. frondosa	*M. Gajdeczka 56*	Colleton Co., SC	FJ985197
G. frondosa	*M. Gajdeczka 57*	Colleton Co., SC	FJ985212
G. frondosa	*R.R. Smith 1563*	Santa Rosa Co., FL	NA
G. frondosa	*W.S. Judd 786*	Norfolk Co., MA	NA
G. nana	*C. van Hoek & B. Wargo 1084*	Polk Co., FL	FJ985225
G. nana	*J. Amoroso 261*	Levy Co., FL	FJ985223
G. nana	*M. Gajdeczka 16*	Alachua Co., FL	FJ985191
G. nana	*M. Gajdeczka 22*	Putnam Co., FL	FJ985202
G. nana	*M. Gajdeczka 23*	Suwannee Co., FL	FJ985203
G. nana	*M. Gajdeczka 31*	Lowndes Co., GA	FJ985206
G. nana	*M. Gajdeczka 38*	Jeff Davis Co., GA	FJ985194
G. nana	*M. Gajdeczka 60*	Alachua Co., FL	FJ985213
G. nana	*M. Gajdeczka 61*	Alachua Co., FL	FJ985214
G. nana	*M. Gajdeczka 64*	Alachua Co., FL	FJ985217
G. nana	*M. Gajdeczka 65*	Alachua Co., FL	FJ985218
G. nana	*M. Gajdeczka 66*	Marion Co., FL	FJ985219
G. nana	*M. Gajdeczka 70*	Sumter Co., FL	FJ985220
G. nana	*R.K. Godfrey 8484583*	Wakulla Co., FL	FJ985222
G. tomentosa	*J.B. Nelson 20500*	Appling Co., GA	FJ985224
G. tomentosa	*B. Tan 698*	Columbia Co., FL	FJ985232
G. tomentosa	*D. Hall 1981*	Clay Co., FL	FJ985231
G. tomentosa	*J.R. Abbott 10463*	Hamilton Co., FL	FJ985234
G. tomentosa	*M. Gajdeczka 17*	Alachua Co., FL	FJ985192
G. tomentosa	*M. Gajdeczka 19*	Putnam Co., FL	FJ985200
G. tomentosa	*M. Gajdeczka 20*	Putnam Co., FL	FJ985201
G. tomentosa × nana	*M. Gajdeczka 21*	Putnam Co., FL	NA
G. tomentosa	*M. Gajdeczka 24*	Suwannee Co., FL	FJ985204
G. tomentosa	*M. Gajdeczka 30*	Lowndes Co., GA	FJ985205
G. tomentosa	*M. Gajdeczka 32*	Lowndes Co., GA	FJ985207
G. tomentosa	*M. Gajdeczka 33*	Lowndes Co., GA	FJ985208
G. tomentosa	*M. Gajdeczka 36*	Coffee Co., GA	FJ985209
G. tomentosa	*M. Gajdeczka 37*	Jeff Davis Co., GA	FJ985193
G. tomentosa × nana	*M. Gajdeczka 43*	Bulloch Co., GA	FJ985210
G. tomentosa	*M. Gajdeczka 58*	Screven Co., GA	FJ985198
G. tomentosa	*M. Gajdeczka 62*	Alachua Co., FL	FJ985215
G. tomentosa	*M. Gajdeczka 63*	Alachua Co., FL	FJ985216
G. tomentosa	*M. Gajdeczka 78*	Marion Co., FL	FJ985221
G. tomentosa	*R.K. Godfrey 83157*	Liberty Co., FL	FJ985230
G. tomentosa	*S. Orzell & E. Bridges 22613*	Lake Co., FL	FJ985233
G. tomentosa	*W.S. Judd 1679*	Beaufort Co., SC	FJ985229
G. ursina	*A. Crooks 526*	Sevier Co., TN	NA
G. ursina	*A. Darr 1909*	Oconee Co., SC	FJ985235
G. ursina	*M. Gajdeczka 47*	Transylvania Co., NC	FJ985195
G. ursina	*M. Gajdeczka 48*	Transylvania Co., NC	FJ985211

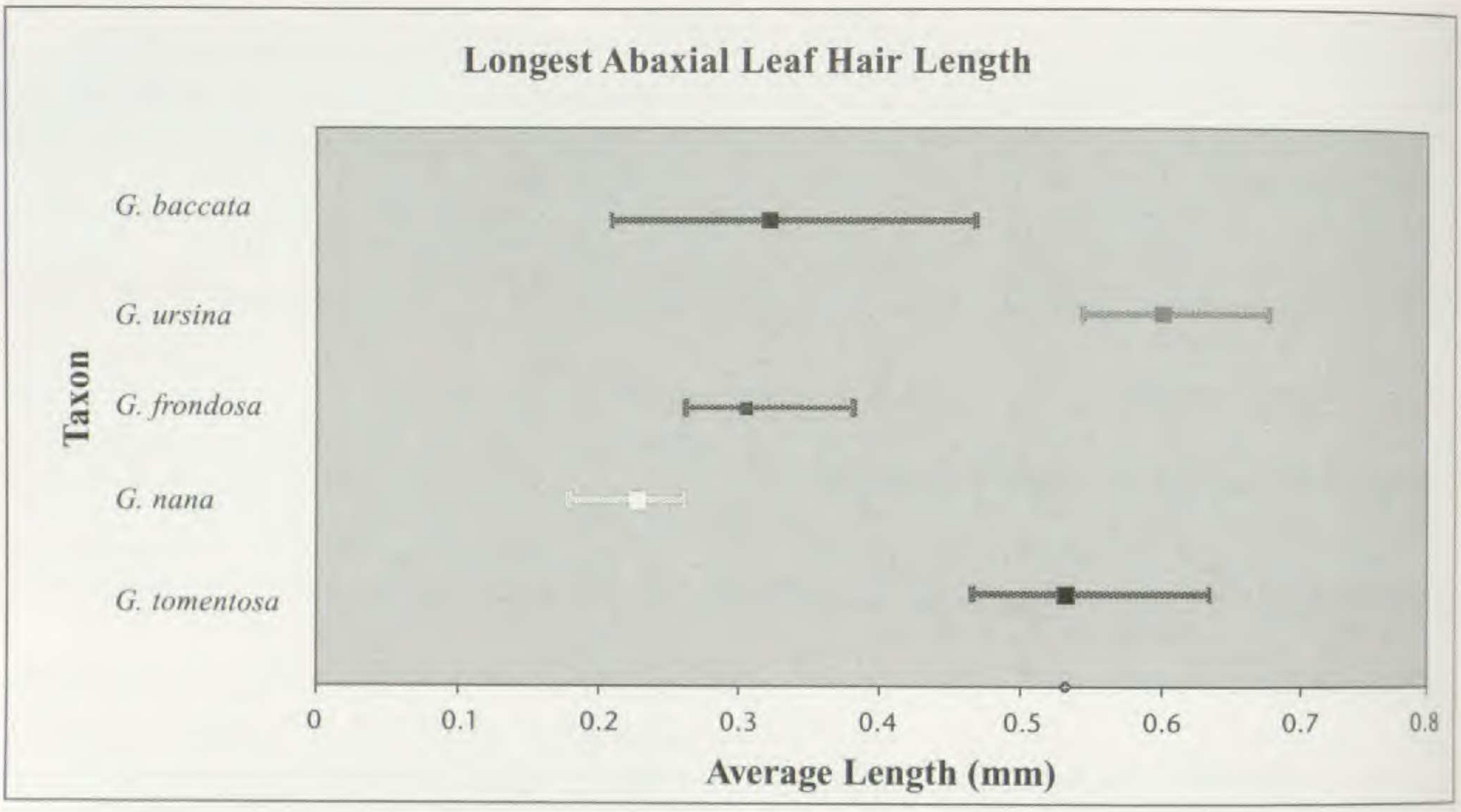

Fig. 2. Graph showing variation in the length of the longest non-glandular hair on the abaxial surface of the leaf for members of *Gaylussacia* sect. *Decamerium*. Squares represent the average of the measurements for all specimens of the corresponding taxon. Lines on either side represent the range of all measurements.

Table 2. Morphological characters and character states included in the phylogenetic analyses of *Gaylussacia*.

1. Plant height: $n < 0.7$ m (0); $n \geq 0.7$ m (1).
2. Ratio of average length of lower secondary branches to length of main stem: $n < 7$ (0); $n \geq 7$ (1).
3. Length of longest non-glandular hair on branch: $n < 0.5$ mm (0); $n \geq 0.5$ mm (1).
4. Number (density) of non-glandular hairs on the adaxial leaf surface per standardized 2.56 mm^2 region: none (0); $0 < n < 10$ (1); $n \geq 10$ (2).
5. Length of longest non-glandular hair on adaxial leaf surface: $0 < n < 0.25$ mm (0); $n \geq 0.25$ mm (1).
6. Length of average non-glandular hair on adaxial leaf surface; $0 < n < 0.175$ mm (0); $0.175 \leq n < 0.322$ mm (1); $n \geq 0.322$ mm (2).
7. Number (density) of non-glandular hairs on the abaxial leaf surface per standardized 1.32 mm^2 region: $0 < n < 6.5$ (0); $6.5 \leq n < 40$ (1); $n \geq 40$ (2).
8. Length of longest non-glandular hair on abaxial leaf surface: $0 < n < 0.275$ mm (0); $0.275 \leq n < 0.45$ mm (1); $n \geq 0.45$ mm (2).
9. Number (density) of resin glands on abaxial leaf surface per standardized 4.0 mm^2 region: $0 < n < 19$ (0); $19 \leq n < 65$ (1); $n \geq 65$ (2).
10. Glaucousness of leaves: none (0); slight (1); moderate (2); strong (3).
11. Length of blade (average of five longest leaves per specimen): $n < 41.1$ mm (0); $41.1 \leq n < 70$ mm (1); $n \geq 70$ mm (2).
12. Width of blade (average of five longest leaves): $n < 20.3$ mm (0); $20.3 \leq n < 30$ mm (1); $n \geq 30$ (2).
13. Petiole length (average of five longest leaves): $n < 1.35$ mm (0); $n \geq 1.35$ mm (1).
14. Bract type: leaf-like (0); small (1; states coded and scored as per Luteyn et al. 1996, and for this character and #16, 17, 18, and 19, our observations, when these were possible, always matched these scorings).
15. Resin glands on adaxial leaf surface: present (0); absent (1).
16. Fruit color: black (0); blue (1; states coded and scored as per Luteyn et al. 1996).
17. Corolla color: white (0); greenish-white to pinkish white (1); red (2; states coded and scored as per Luteyn et al. 1996).
18. Corolla shape: cylindrically urceolate (0); broadly urceolate (1; states coded and scored as per Luteyn et al. 1996).
19. Pilose hairs on the filaments: present (0); absent (1; states coded and scored as per Luteyn et al. 1996).
20. Exudate around perimeter of resin glands on leaves: present (0); absent (1).

TABLE 3. Morphological character state values for terminal taxa used in the phylogenetic analyses. ? = state unknown or "not applicable." All specimens deposited at FLAS

Specimen	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
W. S. Judd 1679	*G. tomentosa*	0	0	1	2	1	1	2	2	1	0	0	0	1	1	1	1	1	0	1	1
M. Gajdeczka 17	*G. tomentosa*	1	1	1	2	1	1	1	2	1	0	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 19	*G. tomentosa*	1	1	1	2	1	2	1	2	1	2	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 20	*G. tomentosa*	1	1	?	2	1	2	2	2	1	?	1	0	1	1	1	1	1	0	1	1
M. Gajdeczka 21	*G. tomentosa* × *nana*	?	1	1	2	1	1	2	2	1	0*	1	0	1	1	1	1	1	0	1	1
M. Gajdeczka 24	*G. tomentosa*	1	1	0	2	1	1	2	2	1	1	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 30	*G. tomentosa*	1	1	1	2	1	1	2	2	1	0	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 32	*G. tomentosa*	1	?	0	2	1	1	2	2	1	0	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 33	*G. tomentosa*	1	?	1	2	1	1	2	2	1	0	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 36	*G. tomentosa*	?	?	1	2	1	1	1	2	1	0	2	1	1	1	1	1	1	0	1	1
M. Gajdeczka 37	*G. tomentosa*	?	0	1	2	1	1	2	2	1	0	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 43	*G. tomentosa* x *nana*	?	1	0	2	1	1	1	2	0	?	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 58	*G. tomentosa*	1	1	1	2	1	2	2	2	1	1	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 16	*G. nana*	1	1	0	1	0	0	1	0	0	?	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 22	*G. nana*	?	0	0	0	0	0	1	0	0	2	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 23	*G. nana*	0	?	0	1	0	0	1	0	1	3	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 31	*G. nana*	1	0	0	1	0	0	1	0	0	2	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 38	*G. nana*	?	0	0	1	0	0	1	0	0	?	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 60	*G. nana*	0	0	0	1	0	0	1	0	0	2	0	0	0	1	1	1	1	0	1	1
M. Gajdeczka 61	*G. nana*	0	?	0	1	0	0	1	0	1	1	0	0	0	1	1	1	1	0	1	1
R. K. Godfrey 8484583	*G. nana*	0	0	0	1	?	?	1	0	0	3	0	0	0	1	1	1	1	0	1	1
A. B. Pittman 05090108	*G. frondosa*	?	?	0	0	?	1	1	1	1	?	1	1	1	1	1	1	1	0	1	1
G. W. Seckinger Jr. 271	*G. frondosa*	?	0	0	0	?	?	1	0	1	?	0	1	1	1	1	1	1	0	1	1
J. B. Nelson 16,473	*G. frondosa*	?	0	?	0	?	?	1	1	1	?	0	0	1	1	1	1	1	0	1	1
M. Gajdeczka 56	*G. frondosa*	1	0	?	0	?	?	1	1	1	2	1	1	1	1	1	1	1	0	1	1
M. Gajdeczka 57	*G. frondosa*	1	0	0	0	1	1	1	1	1	2	1	1	1	1	1	1	1	0	1	1
W. S. Judd 786	*G. frondosa*	1	0	0	1	?	?	1	1	0	1	1	2	1	1	1	1	1	0	1	1
R. R. Smith 1563	*G. frondosa*	1	?	0	1	1	1	1	1	1	0	2	2	1	1	1	1	1	0	1	1
D. Darr 1909	*G. ursina*	?	?	0	1	1	2	0	2	0	0	2	1	1	1	1	0	1	0	0	1
A. Crooks 526	*G. ursina*	?	1	1	0	1	2	0	?	0	1	2	2	1	1	1	0	1	0	0	1
M. Gajdeczka 47	*G. ursina*	1	1	1	0	1	2	0	2	0	0	2	2	1	1	1	0	1	0	0	1
M. Gajdeczka 48	*G. ursina*	1	?	1	1	1	2	0	2	0	0	2	2	1	1	1	0	1	0	0	1
M. Gajdeczka 50	*G. baccata*	1	?	0	1	1	1	0	0	1	?	1	0	1	1	0	0	2	1	0	0
W. S. Judd 716	*G. baccata*	1	1	0	1	1	1	0	1	2	0	0	0	1	1	0	0	2	1	0	0
E. W. Wood 4222	*G. baccata*	?	1	0	1	1	1	0	1	2	0	1	0	1	1	0	0	2	1	0	0
A. Vascott 405	*G. baccata*	?	0	0	1	1	1	0	1	2	0	1	0	1	1	0	0	2	1	0	0
W. S. Judd 1657	*G. baccata*	1	1	0	1	1	1	0	1	2	0	0	0	0	1	0	0	2	1	0	0
M. Gajdeczka 18	*G. dumosa*	0	1	0	1	1	1	0	?	1	0	0	0	0	0	0	0	0	0	0	0

*Collection number represented by three herbarium sheets, and a few leaves do show slight glaucousness.

from some herbarium specimens for DNA extraction. Anticipating a lower DNA concentration, the herbarium extractions were resuspended in only 120 µL of Tris-EDTA (TE) buffer. In all extractions the resuspended DNA was purified using Qiaquick columns (Qiagen, Valencia, California, USA) and Buffer PE, then eluted with Buffer EB.

All PCR reactions were carried out using Sigma Jumpstart *Taq* polymerase and reagents (Sigma-Aldrich, St. Louis, Missouri, USA). Initially a survey of phylogenetically informative regions were carried out using the nuclear ribosomal nrITS region and seven plastid DNA regions (*trnL-F*, *rpl32-trnL*, *trnQ-rps16*, *trnH-psbA*, *atpB-rbcL*, *psbD-trnT* and *trnV-ndhC*) based on suggestions of phylogenetically useful regions in Small et al.

(1998) and Shaw et al. (2005, 2007). Two representative field-collected specimens of each of the three ingroup taxa (*Gaylussacia frondosa*, *G. tomentosa*, and *G. nana*) and one sample each of *G. baccata* and *G. ursina* were included in the survey. The reaction mixture for amplification of nrITS from field collected specimens included 7.0 µL of betaine (5 M), 12 µL H_2O, 2.5 µL 10X buffer, 2.0 µL $MgCl_2$ (25 mM), 0.5 µL dNTPs (10 µM), 0.5 µL of each primer (10 µM), 0.5 µL template, and 0.2 µL *Taq* polymerase (25.5 µL total). In order to improve amplicon concentration, volumes were adjusted to 1.0 µL template and 11.0 µL H_2O (25.7 µL total). Reaction mixtures for the amplifications from herbarium specimens totaled 24.7 µL (18 µL H_2O, 2.5 µL 10X buffer, 2.0 µL $MgCl_2$ (25 mM), 0.5 µL dNTPs (10 µM), 0.5 µL each of forward and reverse primer (10 µM), 0.5 µL template, and 0.2 µL *Taq* polymerase).

The following PCR protocol was used for the initial survey: an initial bake at 94°C for 3 min; then 30 cycles of (1) denaturation at 94°C for 30 s, (2) annealing at 60°C for 30 s, and (3) extension at 72°C for 2 min; and a final extension at 72°C for 3 min. Primers 17SE and 26SE from Sun et al. (1994) and the PCR program in Whitten, et al. (2007) were used for amplification and sequencing of the nrITS region from field collected specimens. With the aim of improving nrITS amplicon yield from herbarium specimens, primers ITS A/ITS C and ITS B/ITS D and the protocol from Blattner (1999) were used, and the reaction mixture was doubled to 50.4 µL (30 µL H_2O, 6 µL $MgCl_2$ (25 mM), 5 µL 10X buffer, 4 µL template 2 µL each of forward and reverse primer (10µM), 1.0 µL dNTPs (10µM), and 0.4 µL Taq polymerase).

Phylogenetic analyses.—Herbarium specimens, based on the first author's own field work or from the collections of FLAS, and representing populations of all the entities within the *Gaylussacia frondosa* complex, were employed as terminal taxa in the phylogenetic analyses. These analyses served as the basis not only of the assessment of phylogenetic relationships within *Gaylussacia* section *Decamerium* but also for hypotheses of species circumscription (based on an application of the phylogenetic/apomorphic species concept; Donoghue 1985; Mishler 1985; Mishler & Theirot 2000).

PAUP* 4.0b10 (Swofford 2002) was used to construct most parsimonious trees for three data sets: (1) a morphological analysis of 38 specimens (i.e., terminal taxa, representing populations; 26 of these field-collected and 12 herbarium/FLAS collections) and 20 characters, and 19 of these parsimony informative (see Table 2), (2) a nrITS analysis of 47 specimens and 827–857bp (31 field-collected specimens) or 648–664 bp (FLAS herbarium material, 16 specimens) but only 12 parsimony-informative sites (and 19 total variable characters), and (3) a combined "total evidence" analysis, i.e., morphology + nrITS, for a pruned data set of 31 specimens (24 field-collected, and 7 herbarium), and 48 variable characters, with 31 of these parsimony informative. All characters were equally weighted and unordered. The morphological analysis used a maximum parsimony (MP) heuristic approach, 500 random-addition replicates, TBR, MaxTree = 200 per replicate, and MulTrees on. Relative support for clades in all analyses was evaluated using a fast-heuristic bootstrap analysis (1000 replicates in the morphological analysis, 100 replicates in the nrITS and combined analyses). The nrITS and analysis used a MP heuristic approach, 100 random-addition replicates, TBR, MaxTree = 10 per replicate, and MulTrees on. The combined analysis was similar but used MaxTree = 10,000. Gaps were coded as missing data; indels were not coded as characters. Morphological character state changes were mapped onto the morphological cladogram and traced onto the total evidence MP cladogram using the PAUP* output in MacClade 4.05 (Maddison & Maddison, 2005). The molecular and morphological analyses resulted in trees with no strongly supported incongruent patterns, so were combined (see "total evidence" analysis, above).

RESULTS

Morphological analysis.—A heuristic search using maximum parsimony yielded 3360 most parsimonious trees (MPTs) with a length of 65 (Consistency Index [CI] = 0.462, Retention Index [RI] = 0.829, Rescaled Consistency Index [RC] = 0.383). The strict consensus tree is shown in Figure 3. *Gaylussacia tomentosa*, *G. nana*, *G. frondosa*, and *G. ursina* form a clade with moderate support (bootstrap [BS] = 78%) in all trees (Fig. 3A). The *G. frondosa* complex, i.e., *G. tomentosa*, *G. nana*, and *G. frondosa*, also forms a clade (BS = 85%; Fig. 3). Within the *G. frondosa* complex, populations of *G. tomentosa* form a clade (without bootstrap support).

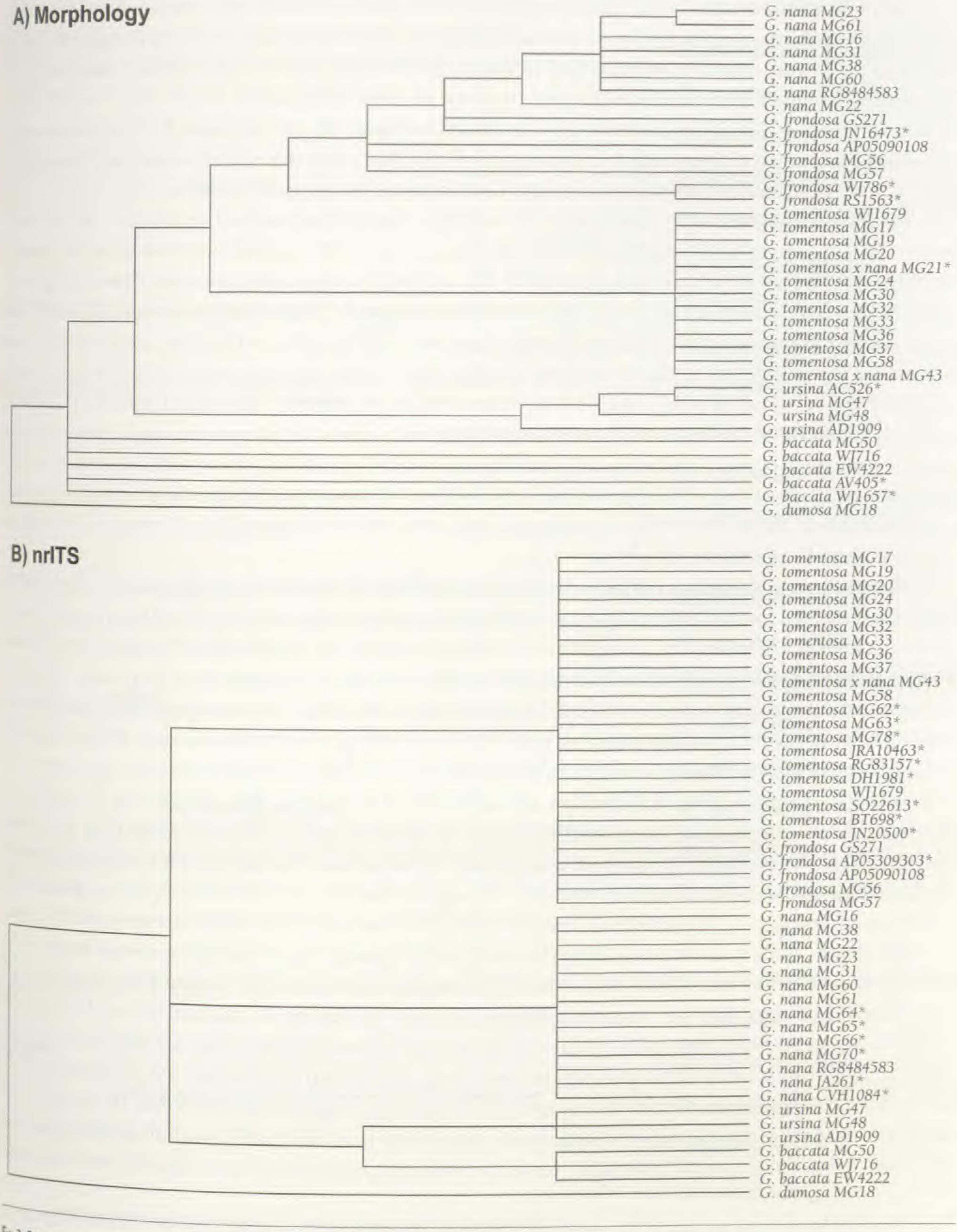

Fig. 3. Strict consensus trees resulting from (A) the morphological analysis and (B) the nrITS analysis. Collector's names abbreviated by initials (see Table 1). OTUs unique to a particular analysis indicated with an asterisk. See results for bootstrap values.

which is sister to a clade comprising the populations of *G. frondosa* and *G. nana* (without bootstrap support). Within the *G. frondosa* + *G. nana* clade, the populations of *G. nana* form a clade (BS = 58%), which is nested within a paraphyletic *G. frondosa*. The terminals representing populations of *G. ursina* also form a clade (BS = 73%), but *G. baccata* is unresolved at the base of the *Decamerium* clade.

nrITS analysis.—None of the plastid regions showed phylogenetically informative variation, so after preliminary work these regions were not pursued. However, the 47 sequences of the nrITS region did show useful variation and, therefore, were studied further. A MP heuristic search (100 replicates, MaxTrees = 10 per replicate) of the nrITS sequences resulted in a total of 1000 MPTs with a length 33 (CI = 0.970, RI = 0.992, RC = 0.962). The strict consensus tree is shown in Figure 3B. The monophyly of *Gaylussacia nana* (BS = 56%) was weakly supported. A *G. tomentosa* + *G. frondosa* clade (BS = 95%) was strongly supported. A third clade in the strict consensus tree groups *G. baccata* and *G. ursina* (BS = 69%).

Combined analysis.—The combined total evidence analysis included all specimens that had both successfully sequenced nrITS regions and morphological data. A MP heuristic search resulted in a total of 1000 MPTs of length 89 (CI = 0.663, RI = 0.878, RC = 0.582). A representative tree is shown in Figure 4, with the strict consensus and bootstrap consensus values mapped. Clades *Gaylussacia nana* (BS = 91%) and *G. tomentosa* + *G. frondosa* (BS = 87%) are strongly supported and are sister in the strict consensus tree, thus supporting the monophyly of the *G. frondosa* complex (BS = 62%). All specimens of *G. tomentosa* form a monophyletic group in the strict consensus tree (but without BS support). Specimen *Gajdeczka 43* (MG43), probably a hybrid (see below), and the operational taxonomic units (OTUs) representing *G. frondosa* form a paraphyletic grade in the *G. tomentosa* + *G. frondosa* clade, and *G. frondosa* forms a clade only in some of the equally parsimonious trees. The *G. tomentosa* + *G. frondosa* + *G. nana* clade is sister to a strongly supported *G. ursina* clade in the SC (BS = 95%). A *G. baccata* clade (BS = 100%) is sister to the *G. ursina* + *G. tomentosa* + *G. frondosa* + *G. nana* clade (BS = 59%).

Morphological synapomorphies.—Mapping morphological characters onto a randomly chosen MPT from the combined (or total evidence) analysis showed that most clades were supported by at least two morphological synapomorphies. The monophyly of *Gaylussacia nana* in the combined MPT was supported by six character states: short non-glandular hairs on both adaxial and abaxial surfaces of the leaf (chars. #5, 6, and 7, Table 2), short and narrow blades (#11 and 12) and short petioles (#13). The monophyly of *G. tomentosa* was supported by dense non-glandular hairs on both adaxial and abaxial leaf surfaces (chars. #4 and 7), as well as long hairs on the branches (#3) and on the abaxial leaf surface (#8). *Gaylussacia tomentosa* specimens also were less glaucous than those of *G. frondosa* and especially *G. nana* (char. #10, see also Fig. 1). *Gaylussacia frondosa* (when monophyletic) was poorly supported by morphological synapomorphies: most *G. frondosa* specimens are characterized by sparse or absent hairs on the adaxial leaf surface (char. #4) and medium-length leaf hairs on the abaxial surface (char. #8), although the variation pattern is homoplasious. In addition, *Gaylussacia frondosa* tends to have fewer unicellular hairs on its twigs than either *G. tomentosa* or *G. nana*.

Specimens of *Gaylussacia ursina* formed a monophyletic group supported by the average length of the non-glandular hairs on the adaxial leaf surface (char. #6) (as opposed to the "longest hair" measurement), long abaxial leaf hairs (#8), the longest and widest blades of all taxa in the analysis (#11 and 12), usually nonglaucous leaves (#10), and sparse glands on the abaxial leaf surface (#9). The monophyly of *G. baccata* was supported by five unique synapomorphies: very dense abaxial leaf glands (char. #9), a red corolla (#17), a cylindrically urceolate corolla (#18), and presence of glands on the adaxial leaf surface (#15). Additional derived features that distinguished it were sparse adaxial leaf hairs (char. #7), medium length abaxial leaf hairs (#8), black coloration of fruits (#16), presence of filament hairs (#19), lack of glaucousness (#10), and narrow leaf blades (#12).

The *Gaylussacia tomentosa* + *G. frondosa* clade was supported mainly by features that differentiate it from *G. nana*: tall plant height (char. #1), long adaxial leaf hairs (#5 and 6), dense abaxial leaf glands (#30), long and wide leaf blades (#11 and 12), and long petioles (#13). The monophyly of the *G. tomentosa* + *G. frondosa* + *G. nana* clade was supported by dense abaxial leaf hairs (char. #30), leaves with slight to intense glaucousness (#10; in many specimens, but lost in some, especially *G. tomentosa*), blue fruits (#16), and glabrous filaments (#19). Finally, the monophyly of the *G. tomentosa* + *G. frondosa* + *G. nana* + *G. ursina* clade was supported by a lack of adaxial leaf glands (char. #15), greenish-white to pinkish-white coloration (#17) of the broadly urceolate corolla (#18), and resin glands lacking exudate (#20).

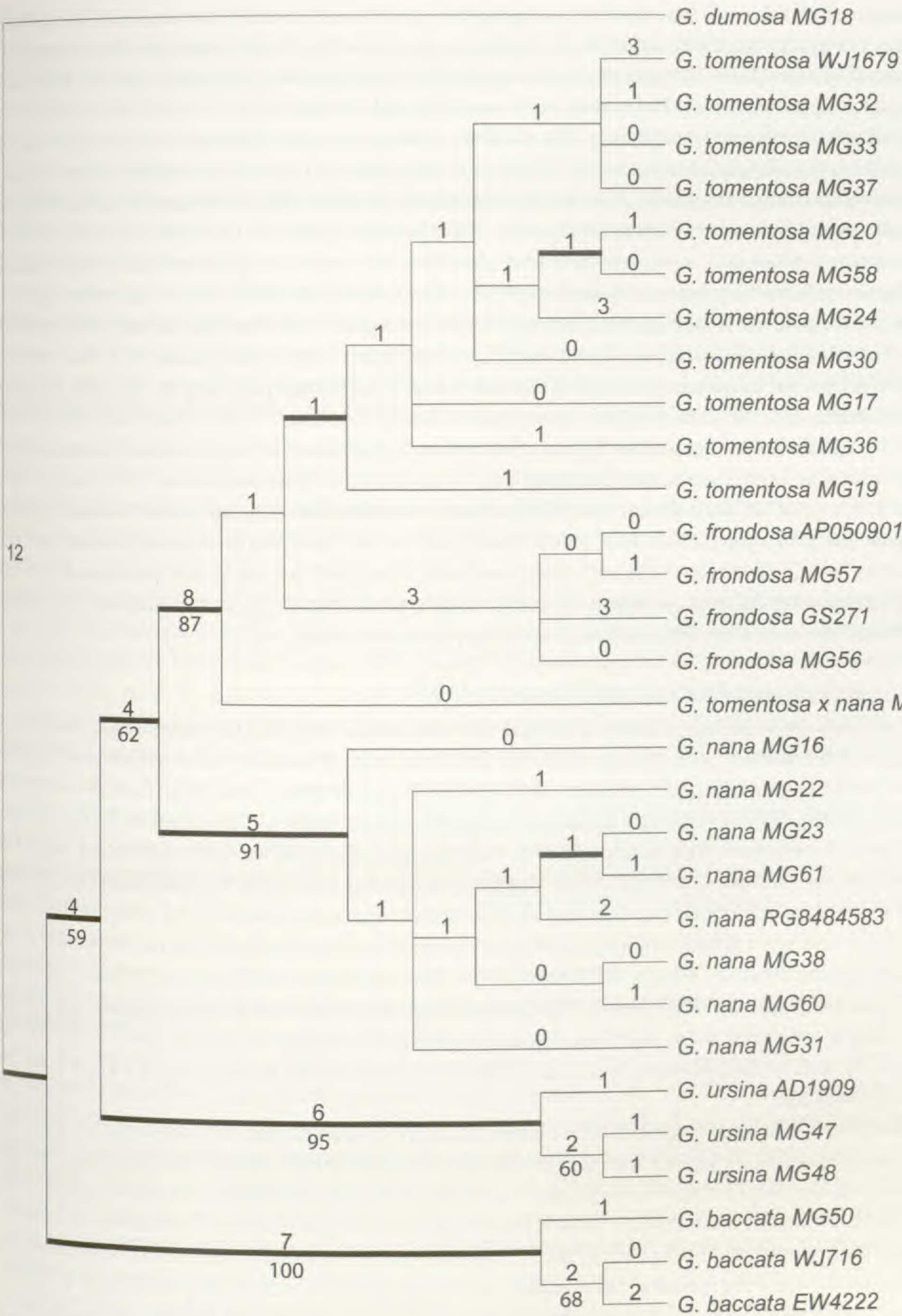

Fig. 4. A representative tree from the combined nrITS and morphology analysis. The numbers above the lines represent the number of character changes. Nodes present in the strict consensus tree are indicated by bold lines; bootstrap consensus values provided below the lines. Individual OTUs indicated by abbreviation of collector names along with the collection number (see Table 1).

Putative hybrids.—Various specimens are putatively hybrids between *G. tomentosa* and *G. nana* (*Orzell & Bridges 19346*, *Herring 1548*, *Gajdeczka 21*, *Gajdeczka 43*, *Gajdeczka 75*, and *Gajdeczka 76*) or between *G. frondosa* and *G. nana* (*Judd 3118*) and all are deposited at FLAS. Most of these specimens were excluded from analysis because of the intermediate nature of the data. The interspecific hybrid status of these specimens is supported by both DNA and morphology. The putative *G. tomentosa* × *nana* hybrids are noteworthy in having leaves that are usually glaucous (and thus similar to *G. nana*) but also have long unicellular hairs on their abaxial surfaces (like *G. tomentosa*); their leaves often are also smaller then is characteristic of *G. tomentosa* (especially so in *Gajdeczka 21*), again like *G. nana*. The specimen *Judd 3118* has small, abaxially glaucous leaves (similar to those of *G. nana*), but their adaxial surfaces are essentially glabrous (like *G. frondosa*), and the twigs are only sparsely pubescent (again, similar to *G. frondosa*). For nrITS, there exist polymorphisms at all of the variable nucleotide positions among these three species. For example, at base 163 in our ITS matrix, there is a variable nucleotide C or T, with C occurring in *G. tomentosa/frondosa* vs. T in *G. nana*; all hybrids exhibit a strong polymorphic signal for both C and T. Additional polymorphic sites (substitutions) are found at sites 207, 344, 358, 679, 682, as well as two indels (insertion/deletion) from bases 704–711 and a single base indel at 739. Because we did not clone these PCR products to separate the different copies of ITS, we cannot be certain of the distribution of these characters and their association with either parental species. However, it has been shown that PCR-mediated recombination can cause false or mixed signal of the original parental types (Lahr & Katz 2009). Nonetheless, we are confident that these sequences represent interspecific hybrids because of the high association of the polymorphic nucleotide positions only at the variable sites among different species and at every variable site among the species in question (see Soltis et al. 2008) and because of the intermediate morphology discussed above.

DISCUSSION

Taking all three analyses into account, it is clear that *Gaylussacia nana* and *G. tomentosa* are cladospecies (Donoghue 1985; Mishler 1985; Mishler & Theriot 2000), i.e., each of these species is a monophyletic group of populations (see Donoghue 1985; Mishler 1985; Mishler & Theriot 2000). Thus, both should be recognized as distinct species. We note that they are largely sympatric and are easily distinguished by the morphological characters listed above. The monophyly of *G. nana* is supported by all analyses, with strong support in the combined analysis (see Fig. 4, BS = 91%). Diagnosable features for *G. nana* included short hairs on both adaxial and abaxial leaf surfaces, short and narrow blades, and short petioles (and compare these with features of *G. tomentosa* above). Recognition of these two species is also justified from the perspective of the diagnostic species concept (Wheeler & Platnick 2000; Davis & Nixon 1992), and the phenetic/taxonomic species concept (Judd et al. 2007; Smith 1994) as evidenced by the data presented in Duncan and Brittain (1966). Finally, they must surely represent distinct evolutionary lineages (de Quieroz 2007; Wiley 1978) as evidenced by their reciprocal monophyly and difference in chromosome number (i.e., $n = 12$ in *G. nana*, $n = 24$ in *G. tomentosa*).

Given that *Gaylussacia tomentosa* and *G. nana* are largely sympatric, and very frequently co-occurring, it is not surprising that we found a few morphologically intermediate specimens—likely hybrids—that also exhibited polymorphic ITS sequences (possibly as a result of their possessing ITS sequences representative of the parental species). The frequency of such plants appears to be quite low (i.e., 7 specimens out of nearly 200 observed); most individuals (both in the field and herbarium) are easily determined. More research is necessary on the breeding system of these plants, and the ploidy levels of both the parental species and the putative hybrids needs to be assessed. Despite the occurrence of these putative hybrids, we believe that *G. nana* and *G. tomentosa* are largely reproductively isolated (possibly by the difference in chromosome number), thus fitting the biological species concept (Mayr 1969).

The results presented here provide strong support for the treatments of Small (1933), Camp (1935, 1941), Wood (1961), Duncan and Brittain (1966) and Luteyn et al. (1996), all of whom distinguished *G. nana* and *G. tomentosa* at the species level. Recognition at the varietal level, as proposed or adopted by Gray (1878),

Chapman (1889), Harper (1906), Radford et al. (1964), Sleumer (1967), and Floyd (2002) is considered inappropriate. A monophyletic *Gaylussacia tomentosa* is present in the nrITS and combined strict consensus trees, albeit without BS support, although the morphological analysis does not resolve *G. tomentosa* into a clade in all trees (see Fig. 3). Twelve morphological characters distinguish *G. tomentosa* from *G. nana* (Table 2, chars. #2–9, 11–13). Five of the characters also separate *G. tomentosa* from *G. frondosa* (chars. #2, 3, 4, 6 and 7); these vary infraspecifically to a greater extent within *G. tomentosa* and *G. frondosa* than in *G. nana*, except branch hair length (char. #3, Table 3). Finally, the difference in ploidy distinguishes *G. tomentosa*, which is the only tetraploid in the subsection (Löve 1976; Luteyn et al. 1996), providing a distinctive apomorphic feature for this taxon.

The taxonomy of *Gaylussacia frondosa* is more problematic. *Gaylussacia frondosa* has no support as a monophyletic group in any of the strict consensus trees, yet the monophyly of this taxon is supported in some of the most parsimonious trees resulting from the combined analysis (Fig. 4). The two character states that could be considered diagnostic for *G. frondosa* are the absence of adaxial leaf hairs except for the midvein and margin (char. #4; Tables 2, 3) and medium-length abaxial leaf hairs (#23). However, their pattern of variation is homoplasious. Thus, *G. frondosa* lacks clear morphological autapomorphies, which likely contributes to its lack of support in the nrITS and morphological analyses. Although placed as a close relative of *G. tomentosa* in the combined analyses, we note that these two taxa can be clearly differentiated by chromosome number, $n = 12$ in *G. frondosa* and $n = 24$ in *G. tomentosa* (Löve, 1976; Luteyn et al., 1996). In addition, *G. frondosa* has a more northern distribution (to NH and MA; Luteyn et al. 1996; Camp 1935) than does *G. tomentosa*, so in large parts of their ranges they are allopatric. It is commonly reported that *G. frondosa* can often reach heights of 2–3 m (Luteyn et al. 1996; Camp 1941), while *G. tomentosa* seldom reaches heights above 1.5 m (Luteyn et al. 1996). Camp (1941) also mentioned the highly clonal habit of *G. tomentosa* and *G. nana*, as opposed to the more spread out, less rhizomatous habit of *G. frondosa*.

Most putative *Gaylussacia tomentosa* × *nana* hybrids were not included in the nrITS analyses due to their polymorphic sequences, however, *Gajdeczka 43* was included (Figs. 3, 4) as its ITS sequence is fairly clear. This specimen (as discussed under results) is morphologically intermediate between *G. tomentosa* and *G. nana* (e.g., it has intermediate-length hairs on the abaxial leaf surface, and it may have been slightly glaucous) and its nrITS sequence shows a slight indication of polymorphism at the variable nucleotide positions (i.e., small secondary peaks are present at the polymorphic sites). The ITS sequence of this specimen was coded using the major peaks at these sites, and thus it was placed in the *G. tomentosa/frondosa* clade (instead of the *G. nana* clade) in the ITS tree (Fig. 3B). Had these sites been coded as polymorphic its position in the cladogram would have been unresolved (within the *G. frondosa* complex). Its more isolated placement in the strict consensus of the combined analysis (Fig. 4), i.e., sister to the remaining members of the *G. tomentosa* + *G. frondosa* clade, results from the interplay between the ITS sequence (with characters arbitrarily coded as those of *G. tomentosa*) and the presence of certain morphological characters of *G. nana*, e.g., shorter hairs on the abaxial leaf surface. *Gajdeczka 43* is placed in the *G. tomentosa* clade (Fig. 3) in the morphology-based analysis due to the more numerous apomorphies of this species (and indeed, this specimen is phenetically most similar to *G. tomentosa*). Another putative hybrid, *Gajdeczka 21*, was included in the morphology-based phylogenetic analysis, and this plant, like *Gajdeczka 43*, was placed in the *G. tomentosa* clade, likely as a result of its expressing more synapomorphies of *G. tomentosa* than *G. nana* (as coded in our matrix). Of course, such placements are influenced by the necessarily somewhat arbitrary delimitations of some of the morphological character states (see methods). The nrITS sequence of *Gajdeczka 21* allows us to confidently hypothesize that this specimen represents a hybrid individual, and its placement with *G. tomentosa* accessions in the morphological strict consensus tree (Fig. 3A) does not cause us to doubt that it is very likely of hybrid origin. Such placements of hybrid individuals are to be expected (McDade 1990, 1992).

Gaylussacia baccata and *G. ursina* were considered outgroups in this study, and as such, their intraspecific taxonomy was not assessed (Floyd 2002). In addition, their species limits have not been controversial. Each is supported as a cladospecies in the total evidence trees (Fig. 4). Strangely, *G. baccata* is paraphyletic in the morphology-based trees (forming the basal branches of the cladogram).

The nrITS analysis resulted in some unexpected results, e.g., *Gaylussacia baccata* and *G. ursina* formed a clade (Fig. 3B), although the resolution of the analysis was too poor to shed light on subsectional relationships, likely as a result of insufficient phylogenetically informative characters. Floyd's (2002) nrITS topology was similarly unexpected (i.e., sect. *Decamerium* was shown as polyphyletic), and her nrITS data was incompatible with her *trnL-trnF* data (as assessed by a partition homogeneity test). Possible lineage sorting or hybridization events affecting the nrITS region might be contributing to the observed irregularities (Floyd 2002), and it should be noted that Floyd coded indels as additional characters, while we did not. Understanding the discrepancies between nrITS sequences and other data sets used for hypothesizing phylogeny in *Gaylussacia* requires additional study.

Our morphological ingroup topography also shows discrepancies with the nrITS and combined topographies, i.e., *Gaylussacia frondosa* groups with *G. nana* (rather than *G. tomentosa*) in the morphological trees. Three character states are synapomorphies of the *G. frondosa* + *G. nana* clade in the morphological tree: habit (#2), short non-glandular hairs on the stems (#3), and moderately dense hairs on the abaxial leaf surface (#7). Floyd's (2002) morphological analysis places a *G. tomentosa* + *G. nana* clade (BS = 73%) sister to *G. frondosa*; *G. tomentosa* and *G. nana* also group together in Floyd's *trnL-trnF* topology. Her analysis, however, did not include any morphological character states that were shared by *G. nana* and *G. frondosa*, but also differed from those of *G. tomentosa*. Our characters #2 and 3 are not treated in Floyd's study, while character #7 is coded similarly for *G. frondosa* (but the state is shared with *G. nana*), and we note that it is not unexpected in analyses involving different evolutionary units to employ different morphological characters, or state delimitations. Also in her analysis, several character states relating to hair density (glabrous twigs, adaxial leaf surfaces and leaf margins, few hairs on abaxial leaf surface) are unique to *G. frondosa*, and thus separate it from *G. tomentosa* and *G. nana*. Twig hair density was measured in our study (and *G. frondosa* does have the least dense hairs), but this character was excluded from our analyses because it follows a similar pattern of variation as other hair density characters. It is possible that glabrous to sparsely pubescent twigs could represent a synapomorphy for the populations of *G. frondosa*.

Finally, the morphological (BS = 85%) and combined (BS = 62%) data support the recognition of a monophyletic *Gaylussacia frondosa* complex (i.e., subsect. *Frondosae*) as it is commonly treated (Camp 1941; Sleumer 1967; Floyd 2002). In the majority rule consensus tree of the nrITS analysis (not shown), a *G. baccata* + *G. ursina* clade is sister to a *G. tomentosa* + *G. frondosa* clade in 52% of trees (with *G. nana* sister to all four species). In Floyd (2002) a *G. tomentosa* + *G. frondosa* + *G. nana* clade was evident in most analyses.

In conclusion, within *Gaylussacia* sect. *Decamerium* subsect. *Frondosae*, it is clear that the largely sympatric *G. nana* and *G. tomentosa* each represent distinct species (regardless of the species concept applied), while the evidence for the distinctiveness of *G. frondosa* is more problematic—although our analyses provide some preliminary evidence that it, too, should be recognized as specifically distinct (Fig. 4). An identification key for these species is available in Luteyn et al. (1996). More work, however, needs to be done to determine relationships within the complex, as the analyses are incongruent in the placement of *G. frondosa*, the representatives of which are more closely related to *G. nana* in the morphological analysis and are more closely related to *G. tomentosa* in the nrITS analysis. We also recommend additional investigations on the ploidy level of members of subsect. *Frondosae*, especially the putative hybrids.

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